REMARKS/ARGUMENTS

Status of the claims and support for the amendments to the claims.

Claims 79 to 84 and 91 to 99 were previously pending and presented for examination. Claims 79 to 81, 91-96, and 99 are herein amended.

Claims 79 to 81 have been amended to replace the recital of a linker that "consists of between 5 amino acids and 50 amino acids" to the recital of a linker that "is from 5 to 50 amino acids in length." These amendments find support *inter alia* in lines 25 to 28 on page 2 of the specification as filed.

Claims 79 to 81, 91 to 96, and 99have also been amended to set forth a linker *polypeptide*. These amendments are supported by the teaching of polypeptide linkers in the specification. For example, lines 25 to 28 on page 2 of the specification as filed and the linkers taught in lines 12 to 19 on page 42 of the specification as filed.

Finally, claim 99 has been amended for clarity and to conform with its antecedents.

In view of the above, the Applicants believe the amendments to the claims add no new matter and respectfully request their entry.

Response to the rejection of claims 79 to 84 and 91 to 99 under 35 U.S.C. §112, first paragraph, for an alleged lack of written description with respect to the recitals of "oxidized and cyclized" and "consists of between 5 amino acids and 50 amino acids".

a. the "consists of between 5 amino acids and 50 amino acids" subject matter.

Without acquiescing on the merits of the rejection and in the spirit of expediting prosecution, the Applicants have adopted the suggestion of the Examiner and amended the claims to substitute "is" for "consists of."

b. the "oxidized and cyclized" subject matter

Applicants submit that the current specification does provide adequate written description for the characterization of amino acids 65 to 67 of SEQ ID NO:2 as oxidized and cyclized. Specifically, Heim *et al.* (Heim *et al.*, *PNAS USA* 91:12501-4(1994), already of

record), incorporated by reference at page 14, line 24 of the current specification, teach in their Abstract:

> The green fluorescent protein (GFP) of the jellyfish Aequorea victoria is an unusual protein with strong visible absorbance and fluorescence from a p-hydroxybenzylidene-imidazolidinone chromophore, which is generated by cyclization and oxidation of the protein's own Ser-Tyr-Gly sequence at positions 65-67. Cloning of the cDNA and heterologous expression of fluorescent protein in a wide variety of organisms indicate that this unique posttranslational modification must be either spontaneous or dependent only on ubiquitous enzymes and reactants. We report that formation of the final fluorophore requires molecular oxygen and proceeds with a time constant (approximately 4 hr at 22 degrees C and atmospheric pO2) independent of dilution, implying that the oxidation does not require enzymes or cofactors.

The above recital accords with the Applicants' disclosure as filed at p. 31, lines 10 to 17, which teaches:

> Recombinant fluorescent protein can be produced by expression of nucleic acid encoding the protein in E. coli. The fluorophore of Aequorea-related fluorescent proteins results from cyclization and oxidation of residues 65-67. Aequorea-related fluorescent proteins are best expressed by cells cultured between about 20°C and 30°C After synthesis, these enzymes are stable at higher temperatures (e.g., 37°C) and can be used in assays at those temperatures.

As the specification directly and via incorporation of the Heim et al. art teaches that residues 65 to 67 of GFP can spontaneously cyclize and oxidize, Applicants submit that persons of ordinary skill in the art would readily recognize that the Applicants were in possession of the subject matter at issue at the time the application was filed. Accordigly, the current specification satisfies the written description requirement of 35 U.S.C. §112, first paragraph, with respect to such subject matter.

Accordingly, Applicants respectfully request that each of the above grounds for rejection be reconsidered and withdrawn.

Response to the first maintained rejection 35 U.S.C. §112, first paragraph (written description)

Claims 79 to 84 and 91 to 99 stand rejected under U.S.C. §112, first paragraph as allegedly failing to comply with the written description requirement. This rejection was principally concerned with the possible breadth of the sequence subject matter given the 85% sequence identity recital and the length of the linker and, more particularly, a concern over whether the linker amino acids are contiguous. Applicants respectfully traverse the rejection. With regard to length of the linker and whether or not the linker polypeptide amino acids are contiguous, the claims now set forth that the linker is between 5 and 50 amino acids in length and, that the tandem proteins are fusion proteins comprising the linker polypeptide. As a fusion protein, the linker polypeptides are necessarily contiguous.

With regard to the breadth represented by the 85% sequence identity recital, the Applicants next provide an analysis which is further informed by the recent revision of the guidelines provided in the U.S.P.T.O.'s Written Description Training Materials (Revision 1, March 28, 2008).

As a threshold matter, the Applicants note that courts have held that the written description requirement imposes no duty to recite known structures in the specification.[1] Accordingly, the knowledge available in the specification *and* the prior art is available to meet the written description requirement. Applicants respectfully draw the Examiner's attention to the analysis of claim 2 in Example 11B, found on pages 41 and 42 of the revised written description training materials published on March 25, 2008 by the USPTO. Applicants presently review the disclosure of the current application with respect to the fact pattern found in example 11B of the revised written description training materials. Applicants will show that the instant fact pattern

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[1] Indeed, a requirement that patentees recite known DNA structures, if one existed, would serve no goal of the written description requirement. It would neither enforce the quid pro quo between the patentee and the public by forcing the disclosure of new information, nor would it be necessary to demonstrate to a person of ordinary skill in the art that the patentee was in possession of the claimed invention. As we stated in *Capon*, "[t]he 'written description' requirement states that the patentee must describe the invention; it does not state that every invention must be described in the same way. As each field evolves, the balance also evolves between what is known and what is added by each inventive contribution." *Id.* at 1358. Indeed, the forced recitation of known sequences in patent disclosures would only add unnecessary bulk to the specification. Accordingly we hold that where, as in this case, accessible literature sources clearly provided, as of the relevant date, genes and their nucleotide sequences (here "essential genes"), satisfaction of the written description requirement does not require either the recitation or incorporation by reference. *Falkner v. Inglis*, 79 USPQ2D 1001 at 1009 (Fed. Cir., 2006).

greatly exceeds that for claim 2 of example 11B and that, accordingly, their specification ***clearly meets*** the written description requirement of 35 U.S.C. §112, first paragraph.

The scope of the pending claims in question is at the broadest a tandem *Aequorea*-related fluorescent protein (herein *Av*GFP-rp), connected through a linker of between 5 and 50 amino acids, in which both fluorescent moieties contain any contiguous sequence of 150 amino acids of the fluorescent protein that has ***at least 85% sequence identity*** with an amino acid sequence from the wild type *Aequorea* green fluorescent protein (***SEQ ID NO:2***) and which differs at least by the substitutions listed in the claims, characterized in that the amino acid residues in both the donor and acceptor fluorescent protein moieties corresponding to positions 65 to 67 of SEQ ID NO:2 are oxidized and cyclized to form a fluorophore, and wherein the tandem protein exhibits fluorescence resonance energy transfer (FRET) when the donor fluorescent protein moiety is excited. This is commensurate with the fact pattern of claim 2 of example 11B, which is described on page 41 as:

> Claim 2 encompasses a genus of nucleic acids that encode the polypeptide of ***SEQ ID NO:2*** and those that encode any polypeptide having ***85% structural identity*** to SEQ ID NO:2, wherein the polypeptide additionally ***has activity Y***.

As such, both claims set forth an element reciting ***85% identity*** to a ***SEQ ID NO*** to a polypeptide with a ***given activity***, which in the instant application is FRET.

In example 11B of the training materials the hypothetical specification discloses "*the reduction to practice of only* ***a single species*** *that encodes SEQ ID NO:2 and has activity Y.*" This is contrasted to the instant specification, which discloses the reduction to practice of ***twelve species*** embraced by the pending claims in the specification. Specifically, Table 2, found at page 21 of the current specification, provides fluorescent data for 8 tandem constructs that satisfy the requirements of the pending claims. Example 2 of the Applicants' specification, found on pages 43 to 45 of the specification as filed, describes the reduction to practice of 4 additional tandem proteins satisfying the requirements of the pending claims. Accordingly, the number of species disclosed in the instant application ***exceeds*** that of example 11B, by twelve-fold.

Furthermore, in the fourth paragraph on page 41 of the written description training materials, it is disclosed that example 11B includes:

> ***...no other drawings or structural formulas*** disclosed of a nucleic acid that encodes either (i) SEQ ID NO:2 or (ii) a polypeptide with 85% sequence identity to SEQ ID NO:2 wherein the polypeptide also has activity Y.

This is in stark contrast to the instant application, which teaches the high-resolution crystal structure of GFP by incorporating by reference U.S. Patent Application Serial No. 08/706,408 at page 14, line 27 of the current specification. The '408 application provides the high-resolution crystal structure of GFP in Figure 1, the corresponding crystallographic coordinates in Figure 5, a stereo-diagram of the chromophore and surrounding residues with an d without electron density in Figures 2A and 2B, and a schematic diagram of the first and second spheres of coordination of the chromophore in Figure 2C (see, U.S. Patent No. 6,124,128, already of record and which matured from U.S. Patent Application Serial No. 08/706,408). As such, the structural information provided by the instant application far ***exceeds*** that provided in example 11B.

The fifth paragraph on page 41 of the written description training materials discusses the scope of the claim with respect to the knowledge in the art, and is reproduced here for the Examiner's convenience:

> The disclosure of SEQ ID NO:2 combined with the knowledge in the art regarding the genetic code would have put one in possession of the genus of nucleic acids that encode SEQ ID NO:2. Further, with the aid of a computer, one could list all of the nucleic acid sequences that encode a polypeptide with at least 85% sequence identity to SEQ ID NO:2.

In accordance with the above example, Applicants could list all of the polypeptides with at least 85% sequence identity to SEQ ID NO:2 with the aid of a computer. Further, Applicants point out that due to the degeneracy of the genetic code, that the genus of nucleic acids encoding a polypeptide with 85% sequence identity to a SEQ ID NO:2 is ***several orders of magnitude*** larger than the genus of polypeptides with 85% sequence identity to a SEQ ID NO:2. Accordingly, the claimed genus in the instant

application is several orders of magnitude ***smaller*** than the genus claimed in example 11B.

Finally, in the last two paragraphs of example 11B starting on page 41 of the written description training materials, the correlation between structure and function is addressed:

> ...the specification fails to teach which of the nucleic acid sequences that encode a polypeptide with at least 85% sequence identity to SEQ ID NO:2 encode a polypeptide having the required activity Y.
>
> Nonetheless, the specification identifies two domains responsible for the activity Y, i.e., a binding domain and catalytic domain. The specification also predicts that conservative mutations in these domains will result in a protein having activity Y. Although all conservative amino acids substitution in these domains will not necessarily result in a protein having activity Y, those of ordinary skill in the art would expect that many of these conservative substitutions would result in a protein having the required activity. Further, amino acid substitutions outside of the two identified functional domains are unlikely to greatly affect activity Y. Thus, a correlation exists between the function of the claimed protein and the structure of the disclosed binding and catalytic domains. Consequently, there is information about which nucleic acids can vary from SEQ ID NO:1 in the claimed genus of nucleic acids and still encode a polypeptide having activity Y. Based on the applicant's disclosure and the knowledge in the art, those of ordinary skill in the art would conclude that the applicant would have been in possession of the claimed genus of nucleic acids based on the disclosure of the single species of SEQ ID NO:1.

In the above example, for which a crystal structure was not disclosed, the specification identifies two functional domains and predicts that conservative mutations would retain activity Y. In contrast, the instant specification, which does disclose the 3-dimentional structure of GFP, teaches not only the functional domain, the chromophore and surrounding residues as diagramed in figures 2A-C of U.S. Patent No. 6,124,128, but teaches the specific spectral effects caused by a number of mutations in Table A found at page 16 of the instant specification, teaches that the efficiency of FRET can be adjusted by altering the intermolecular interactions between the two fluorophores in the two full paragraphs found on page 19 of the specification, and subsequently teaches 17 residues

that can be mutated to accomplish just this in the first two paragraphs found on page 20 of the specification. Additionally, the specification teaches 12 different protease recognition sequences that can be used in the linker region in Table 3, found on page 24 of the specification, as well as methods of finding cleavage recognition sites for proteases with unknown specificity in the first paragraph of page 25 of the specification.

Further, due to the β-barrel fold of GFP, a large number of residues are found in the 11 loops connecting the individual strands and an similarly large number of residues have side chains that are surface exposed. Applicant's submit that these residues do not directly contribute to the electron environment of the chromophore and that one of ordinary skill in the art would appreciate that these residues could be expected to be mutated with little to no consequence. Accordingly, Applicants submit that the combined teachings of the crystal structure of GFP, the fluorophore active site of the molecule, the spectral properties of mutational combinations in Table A, and the teaching that no less than 17 specific residues can be mutated to alter the efficiency of FRET in the tandem molecule establishes a correlation between structure and function that far ***exceeds*** the correlation described in example 11B.

The above comparison of the fact patterns for the instant specification and example 11B in the written description training materials clearly demonstrates that the disclosure of the instant application far ***exceeds*** that of example 11B. Specifically, both claims recite ***85% identity*** to a ***SEQ ID NO*** with a ***given activity***, the number of species disclosed in the instant application ***exceeds*** that of example 11B by twelve-fold, the claimed genus in the instant application is several orders of magnitude ***smaller*** than the genus claimed in example 11B, and the correlation between structure and function for the instant application far ***exceeds*** the correlation described in example 11B. As the disclosure of the instant application is equal to or exceeds that found in example 11B and the conclusion found at the end of example 11B, on page 42 of the written description training materials, is that "*(t)he specification **satisfies** the written description requirement of 35 U.S.C. 112, first paragraph, with respect to the scope of claim 2*", the instant specification ***must*** surely satisfy the written description requirement of 35 U.S.C. §112, first paragraph, with respect to the scope of claims.

In response to the Examiner's enablement allegations that "*the claims encompass other mutations not defined which could result in a structure that would not produce FRET*" (*see*, Office Action of February 20, 2008, at lines 9 to 11 of page 4), Applicants respectfully disagree. The pending claims clearly set forth the requirement "*...wherein said donor fluorescent protein moiety and said acceptor fluorescent protein moiety exhibit fluorescent resonance energy transfer when said donor fluorescent protein moiety is excited...*". As such, the claims clearly ***do not*** encompass any mutation that could result in a structure that would not produce FRET. Moreover, pursuant to MPEP § 2164.08(b), even if present, the presence of inoperative embodiments within the scope of a claim does not necessarily render a claim nonenabled. The standard is whether a skilled person could determine which embodiments that were conceived, but not yet made, would be inoperative or operative with expenditure of no more effort than is normally required in the art. Atlas Powder Co. v. E.I. du Pont de Nemours & Co., 750 F.2d 1569, 1577, 224 USPQ 409, 414 (Fed. Cir. 1984) (prophetic examples do not make the disclosure nonenabling).

In response to the Examiner's statement that "*no tables were found on pages 21 or 24*" of the specification (*see*, Office Action, at the final paragraph on page 11), Applicants respectfully referenced the pagination of the specification as filed and recorded in PAIR under the date of 01-25-2002, where Tables II and III can be found on pages 21 and 24 respectively. Applicants note that perhaps the Examiner was viewing the specification as a pdf file wherein the title page was shown as page 1 of the pdf document; however, this page is not considered page 1 of the specification as filed. Otherwise, the Applicants have no explanation for the difference in page numbers.

Accordingly, Applicants respectfully request that the above grounds for rejection be reconsidered and withdrawn.

Response to the first maintained rejection 35 U.S.C. §112, first paragraph (written description)

Claims 79-81, 85-94, 97, and 99 stand rejected under U.S.C. §112, first paragraph as allegedly failing to comply with the enablement requirement. Applicants respectfully traverse the rejection.

Without acquiescing on the merits of the rejection and in the spirit of expediting prosecution, the Applicants have amended independent claims 79 to 81 to set forth a linker *polypeptide* rather than a linker *moiety.* The Examiner's rejection was predicated upon the contention that the specification as filed at p. 8, lines 12 to 15, defined a *linker moiety as* embracing a fluorescent moiety. Without acquiescing on the merits and in order to expedite prosecution of the instant application, the Applicants would amend each of the claims as needed to avoid the disputed term. Applicants also note that the pending claims require that the linker moiety is between 5 and 50 amino acids, and thus do not encompass linkers shorter than 5 residues, or longer than 50 residues.

To further address specific concerns set forth in the prior Action, the Applicants now proceed to supplement their earlier *Wands* analysis by providing additional remarks concerning three of the *Wands* factors most at issue: the *Teachings of the Specification*, the *State of the Art*, and the *Amount of Experimentation* required to practice the invention.

Teachings of the Specification:

As outlined in the previous amendment dated December 5, 2007, the specification teaches how to generate a variant of an *Av*GFP-rp which contains up to 38 mutations (84% sequence identity), with respect to the wild type protein sequence, and which still retains useful, if not improved, fluorescent activity. This alone is enough for any person skilled in the art, which in this case would include protein design and evolution, to practice the full scope of the invention. Additionally, as discussed in previous amendment, the specification teaches how to readily generate and select fluorescent proteins with desired spectral properties according to the claims, through the incorporation of several references that do exactly this.

The teaching of the high-resolution crystal structure of GFP in the instant specification facilitates the selection of additional mutations that would not significantly alter the fluorescent properties in a variant of an *Av*GFP-rp. The provided structure and description of its

functional domains serves to identify regions which may be individually modified to achieve distinct purposes without an expectation of affecting the functioning of other domains. It also identifies individual residues and their environments which further provides a person of ordinary skill in the art with opportunities to conservatively substitute residues with little expectation of greatly altering the activity of a GFP. Applicants respectfully submit, that any structural biologist of average skill in the art, would readily be able to generate a plethora of silent mutations in a variant of an *Av*GFP-rp, through manual inspection of the crystal structure alone. This is especially true for residues distal to the chromophore, those whose side chains are solvent exposed and do not contribute directly to the electron environment of the chromophore, and those found in loop regions connecting the beta-strands in the ternary structure.

As evidenced by the crystal structure of GFP, these classes of residues comprise well over 25% of the GFP protein. As such, even without the teachings of the up to 38 specific mutations in the specification, which alone are more than sufficient, the crystal structure suggests more than a sufficient number of conservative mutations that can be made with little to no consequences. Accordingly, Applicants submit that the teaching of the specification is far more than sufficient to enable to the use of the full scope of the invention as claimed.

State of the Art and the Amount of Experimentation Considered Routine:

The state of the art and the amount of experimentation are inter-related. The state of the pertinent experimental methodology is sufficiently advanced that what might otherwise be an enormous amount of experimentation is merely routine. As discussed above, the directed evolution and screening methodologies employed in the field of fluorescent proteins are both routine and rather simple. When these methodologies are coupled with the abundance of biochemical and biophysical knowledge available for these proteins, as further supported by the teachings of the specification, and the ready availability of many simple mutagenesis and general cloning techniques, also evidenced in the specification, the generation of a vast number of mutations in any fluorescent protein, let alone the mere 36 that are required to practice the full scope of the claimed invention, becomes routine.

Most importantly, a principal premise of the Action is that one of ordinary skill would not be able to create a protein with a diverse combination of mutations. However, whatever may be the general validity of this premise cited by the Examiner the cited premise has been amply demonstrated to not apply to GFP. The capabilities in this art are exemplified by the work of Campbell et al. who engineered 33 mutations into a GFP-related protein isolated from *Discosoma coral*, dsRed, without the aid of vast knowledge, as provided for *Av*GFP in the instant application and these remarks. This clearly shows that the amount of experimentation required to practice the claimed invention is well within the capacity of those in the broader field (Campbell et al., PNAS 99:7977-82, 2002, already of record, see Abstract). The Campbell et al. mutant had about 85% sequence homology to the wild type dsRed protein and retained useful fluorescent activity, including advantageous shifts in its emission and excitation peaks, with respect to the wild type protein.

The ability of persons of ordinary skill in the art to re-engineer GFP has been demonstrated in fact (see, Lawrence et al., J. Am. Chem. Soc. 129:10110-10112 (2007), enclosed with IDS). Starting from a modified GFP which has about 7 substitutions already from the wild-type GFP, they re-engineered several GFP's having up to an additional 36 amino acids ***non-conservatively*** substituted (i.e., negatively charged or neutral amino acids were replaced by an Arg or Lys). In another of the modified GFP proteins, 15 negatively charged or neutral amino acids were replaced by a Glu or Asp. Over all, modified GFPs differing in overall charge from +48 to -30 were made and found to have fluorescence activity. These results clearly show that the GFP is extremely tolerant to substitutions and highly diverse variants can be readily obtained by persons of ordinary skill in the art.

In view of the extensive disclosure of compatible mutations in the specification, the crystallographic and functional analyses provided therein, the advanced state of the art with respect to the manipulations needed to practice the claimed invention, the relative simplicity with which mutants may be screened in truly enormous numbers, and the ***demonstrated*** ability of others *in this field of art* to obtain useful fluorescent proteins with less than 85% sequence identities, the Applicants submit that the claimed invention can be practiced with an amount of experimentation which is *not* undue but, rather, routine in the art.

In response to the Examiner's allegation that "the specification does not provide guidance as to covalent binding occurring via cyclization and oxidation of amino acids of the donor and acceptor protein moieties, or via any other methods considered to produce the "coupling of the donor and acceptor protein moieties", Applicants respectfully point out that the currently pending claims include the proviso that "*the donor moiety, the linker polypeptide, and the acceptor moiety are fused in a single amino acid sequence*". Thus, the only embodiments embraced by the currently pending claims, are tandem proteins linked through amino acids contained in the same polypeptide, *i.e.* translated from a single polynucleotide or synthesized in a contiguous fashion. Applicants respectfully point out that the specification teaches this "coupling" method consistently throughout the application, for example, in Figure 2, in lines 28 to 30 on page 2 of the specification as filed, in lines 15 to 17 on page 29 of the specification as filed, in the paragraph bridging pages 31 and 32 of the specification as filed, and as all of the compositions generated in Examples 1 and 2 of the specification.

Accordingly, in view of the ample evidence now provided, the Applicants respectfully request that the above grounds for rejection be reconsidered and withdrawn.

Response to the rejection of claims 79-84 and 91-99 under the judicially created doctrine of obviousness-type double patenting over claims 1-13 and 43-44 of U.S. Patent No. 6,803,188

Without acquiescing on the merits, in the event that claims are allowed in this application and a terminal disclaimer must be provided, Applicants would submit a Terminal Disclaimer in accordance with 37 C.F.R. 1.132(c). Applicants therefore request that the rejections of be held in abeyance until such time as a claim is otherwise deemed to be in condition for an allowance.

CONCLUSION

In view of the foregoing, Applicants believe all claims now pending in this Application are in condition for allowance. The issuance of a formal Notice of Allowance at an early date is respectfully requested.

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at 925-472-5000.

Respectfully submitted,

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